J. Endocr. (1973), 58, 21–30
With 4 plates
Printed in Great Britain

ACCUMULATION OF NICOTINE IN PANCREATIC ISLETS AND CALCITONIN-PRODUCING CELLS IN MICE AND CHICKS DEMONSTRATED BY MICRO- AND WHOLE-BODY AUTORADIOGRAPHY

PREMYSL SLANINA AND HANS TJÄLVE

Department of Pharmacology, Royal Veterinary College, Stockholm, Sweden, and Department of Toxicology, University of Uppsala, Sweden

(*Received* 5 *October* 1972)

SUMMARY

By autoradiographic methods, nicotine was shown to be specifically accumulated in the pancreatic islets in mice. The results also indicated a high accumulation of nicotine in the parafollicular cells of the thyroid in mice and an accumulation was also shown in the ultimobranchial glands in chicks. Like the parafollicular cells of the thyroid in mammals, the ultimobranchial glands of birds are known to produce calcitonin. Metabolic studies with nicotine *in vitro* and autoradiographic studies with the main nicotine-metabolite cotinine, indicated an accumulation of unchanged nicotine (not metabolites) in the cells.

The results are discussed in view of the fact that biogenic amines have been shown to be operative in these endocrine organs. It is suggested that nicotine can share common transport and/or storage mechanisms with biogenic amines in the cells. An effect of nicotine on hormone storage and/or release may take place via an interference with aminergic mechanisms in the cells.

INTRODUCTION

Nicotine may exert pharmacological effects on a number of different tissues such as the adrenal medulla, the hypophysis, and sympathetic and parasympathetic ganglia (see Volle & Koelle, 1970). Nicotine has also been shown to accumulate in tissues where the effects are exerted. This has been found to be the case in the adrenal medulla, the hypophysis and the superior cervical ganglion (Hansson & Schmiterlöw, 1962; Appelgren, Hansson & Schmiterlöw, 1963; Brown, Hoffmann & Roth, 1969).

The adrenal medulla and the hypophysis have been classified among a group of endocrine polypeptide hormone-secreting cells called the APUD series (amine precursor uptake and decarboxylating series) (for review see Pearse, 1969). (The polypeptide suggested to be produced by the adrenal medulla is chromogranin (Smith &

Winkler, 1967; Pearse, 1969).) To this group of cell systems also belong the pancreatic islets, the calcitonin-producing parafollicular cells of the mammalian thyroid (Bussolati & Pearse, 1967; Kracht, Hachmeister, Brevstedt, Bönicke & Lenke, 1968) and the ultimobranchial gland (UBG) (Pearse, 1969) which is known to produce calcitonin in birds, amphibians and fishes (Hirsch & Munson 1969; Copp, 1969). The cells of the APUD series share several common characteristics, such as the ability to synthesize and store dopamine and serotonin (see, for example, Falck, Larsson, von Mecklenburg, Rosengren & Svenaeus, 1964; Ritzén, Hammarström & Ullberg, 1965; Cegrell, 1968; Almquist, Malmquist, Owman, Ritzén, Sundler & Swedin, 1971; Tjälve & Slanina, 1971), and the presence of a strong positive reaction for pseudo- or true cholinesterase (Coupland, 1958; Pearse, 1969; Almquist *et al.* 1971).

The present study was performed to investigate whether nicotine is accumulated in the pancreatic islets, the parafollicular cells of the thyroid and the cells of UBG. Such an accumulation may indicate an effect of nicotine in these cells analogous to the case in the adrenal medulla, the hypophysis and the superior cervical ganglion. An investigation was also performed to see if cotinine, a major nicotine metabolite (McKennis, 1960; Hansson & Schmiterlöw, 1962; Stålhandske, 1970), is accumulated in the pancreatic islets or the parafollicular cells. In addition an in-vitro study of nicotine metabolism in the pancreas was undertaken.

MATERIALS AND METHODS

Labelled substances. The labelled compounds used were nicotine [*N*-methyl-^{14}C] bitartrate (sp. act. 24 mCi/mmol), [^{3}H]nicotine (generally labelled; sp. act. 324 mCi/mmol) and [2-^{14}C]I-cotinine (sp. act. 35 mCi/mmol). The labelled nicotine compounds were obtained from the Radiochemical Centre, Amersham, England. [2-^{14}C]-Cotinine was kindly donated by Dr Herbert McKennis, Jr, Richmond, Va., U.S.A.

Experimental animals. Male albino mice of the NMRI strain weighing 20 g were used. They were fed a standard pellet diet (Ewos, Sweden) and received water *ad libitum*.

For the distribution studies in chicks, male White Leghorn 16-day-old chickens (weight about 130 g) were used. The birds were fed a standard growth diet (Forss, Sweden) and had free access to drinking water.

Whole-body autoradiography. [^{14}C]Nicotine was injected into a tail vein in each of five mice at a dose of 10 μCi/animal (calculated as 3·4 mg/kg). The survival times were 5 min, 15 min, 30 min, 1 h and 2 h. Two mice were also given an i.v. injection of 10 μCi cotinine (corresponding to 2·5 mg/kg) and were killed 5 and 30 min after the injection.

Three chicks were each given an injection into a radial vein of 14 μCi [^{14}C]nicotine (corresponding to 0·6 mg nicotine/kg). The chicks were allowed to survive for 5 min, 30 min and 2 h respectively.

After the survival periods stated above the animals were anaesthetized with ether and killed by immersion in a mixture of hexane and solid CO_2 (−78 °C). Then they were embedded in carboxymethylcellulose mixed with water. Saggital sections (20 or 30 μm thick) of the whole frozen animals were cut and dried at −15 °C according to Ullberg's autoradiographic technique by which each section is mounted on tape (no. 810 Minnesota
tape-mounted sections we
in a press at −15 °C. Afte
films were developed and
For semiquantitative eval
different organs was com
^{14}C-isotope standard (Berl

Microautoradiography.
mice at a dose of 0·25 mCi
kg). The survival periods
experiments were 5 and 3
time interval) and speci
removed and immediatel
specimens were then freez
and embedded in paraffin
tape (Minnesota Mining a
photographic plates (G5
microautoradiographic te
for isotopes in water-solubl
the tape was removed in
sections were stained witl

Metabolism of [^{14}*C*]*nicot*
(as a reference) weighing
in Krebs–Henseleit phos
Schmiterlöw (1964). The
After incubation the slice
with chloroform:methanc
Schmiterlöw, 1969). The
mined in a Packard Tri-C
of the added activity in va
phase was subjected to th
using ethanol:acetone:be
Radioactive compounds
X-ray film for about 4 wee
on the plates were scrape

Auto

Mice

On the whole-body au
marked accumulation of
accumulation was seen at
then declined a little at 1
activity was comparable t
The microautoradiogra

tape (no. 810 Minnesota Mining and Manufacturing Co.) (Ullberg, 1954, 1958). The tape-mounted sections were pressed against X-ray film (Kodirex; Kodak) and stored in a press at −15 °C. After the exposure time, varying from 2 weeks to 2 months, the films were developed and the sections were stained with haematoxylin and eosin. For semiquantitative evaluation of whole-body autoradiograms the radioactivity in different organs was compared with autoradiograms of a simultaneously exposed ^{14}C-isotope standard (Berlin & Ullberg, 1963).

Microautoradiography. [^{3}H]Nicotine was injected into a tail vein in each of six mice at a dose of 0·25 mCi in 0·2 ml distilled water (corresponding to 0·25 mg nicotine/kg). The survival periods based upon the results of the whole-body autoradiographic experiments were 5 and 30 min. The mice were killed by decapitation (three at each time interval) and specimens from the pancreas and the thyroid were quickly removed and immediately frozen in isopentane cooled with liquid nitrogen. The specimens were then freeze-dried under vacuum (10^{-4}/mmHg) at −40 °C for 4 days and embedded in paraffin wax *in vacuo*. Sections, 5–7 μm thick, were mounted on tape (Minnesota Mining and Manufacturing Co, U.S.A., Tape no. 688), fastened to photographic plates (G5 and K2, Ilford) and exposed at −20 °C according to the microautoradiographic technique of Hammarström, Appelgren & Ullberg (1965) for isotopes in water-soluble form. After the exposure (which varied from 1 to 3 weeks) the tape was removed in xylol and the photographic plates were developed. The sections were stained with haematoxylin–eosin.

Metabolism of [^{14}*C*]*nicotine* in vitro. Tissue slices of the pancreas and the liver (as a reference) weighing together 150 mg were incubated with 0·5 μCi [^{14}C]nicotine in Krebs–Henseleit phosphate buffer for 2 h according to Hansson, Hoffman & Schmiterlöw (1964). The tissues from four mice were used in a separate incubation. After incubation the slices were homogenized and the homogenate was extracted with chloroform:methanol (2:1, v/v) (Stålhandske, Slanina, Tjälve, Hansson & Schmiterlöw, 1969). The radioactivity obtained in the different phases was determined in a Packard Tri-Carb liquid scintillation counter. Combined mean recoveries of the added activity in various fractions were between 80 and 85 %. The chloroform phase was subjected to thin-layer chromatography on silica-gel plates (Stahl, 1962) using ethanol:acetone:benzene:conc. NH_4OH (5:40:50:5, by vol.) as a solvent. Radioactive compounds were located by exposing the plate to Kodak No-screen X-ray film for about 4 weeks. After the development of the film the appropriate spots on the plates were scraped off and the activity was determined.

RESULTS

Autoradiographic studies with [^{14}*C*]*nicotine*

Mice

On the whole-body autoradiograms of the mice injected with [^{14}C]nicotine, a marked accumulation of radioactivity could be seen in the pancreatic islets. The accumulation was seen at 5 min (Pl. 1, fig. 1), it was high up to 30 min (Pl. 1, fig. 2), then declined a little at 1 h and disappeared after 2 h. The concentration of radioactivity was comparable to that seen in the adrenal medulla.

The microautoradiographic studies with [^{3}H]nicotine (Pl. 2, fig. 3) revealed a

rather uniform distribution of radioactivity throughout the islet with most of the cells being labelled. Labelling could be seen over both the nucleus and the cytoplasm of the cells. The radioactivity in the exocrine pancreas was considerably lower than in the islet.

In the thyroid an accumulation of radioactivity could be observed from 5 min to 1 h after the injection of [^{14}C]nicotine, similar to the pancreatic islets. The highest accumulation in the thyroid was confined to some spots scattered over the gland (Pl. 3, fig. 4).

The microautoradiograms of the thyroid showed that the labelling was mainly concentrated in the cells situated in or between the follicular walls (Pl. 3, fig. 5) with a distribution pattern resembling that of the parafollicular cells.

Chicks

The results mentioned above indicated that nicotine accumulated in the region of the mouse thyroid where there are parafollicular 'C' cells, known to produce and store calcitonin in mammals. In birds the UBG are known to be the main source of calcitonin, thus corresponding functionally to the 'C' cells of mammals (see Introduction). The extrathyroidal localization of the UBG in birds (Garlich, 1971) renders them especially suitable for our studies as it makes it possible to compare directly the accumulation of label in the thyroid and the UBG on the same whole-body autoradiogram.

The whole-body autoradiograms of the chicks showed a high uptake of radioactivity in the UBG 5 and 30 min after the injection of [^{14}C]nicotine (Pl. 4, fig. 6). The radioactivity exceeded that in the blood by about 2–4 times. Two hours after the injection no labelling was detectable. The thyroid, on the other hand, showed a concentration of radioactivity which corresponded to or was only slightly higher than that of the blood within 30 min after the injection. The radioactivity disappeared after 2 h.

Thus from the present results there is a strong indication that the cells which produce and store calcitonin are also able to take up and accumulate nicotine.

Metabolic studies

Nicotine has been found to be rapidly metabolized by various organs in mice (Hansson *et al.* 1964; Stålhandske, 1970). To see if metabolism of nicotine also takes place in the pancreas, an in-vitro study of the metabolism of [^{14}C]nicotine in pancreatic slices was carried out.

Incubation of [^{14}C]nicotine with pancreatic slices showed that the pancreas of the mouse lacks the ability to metabolize nicotine to any appreciable extent. After the extraction of the incubation mixture with chloroform:methanol (2:1, v/v) there was almost total recovery of radioactivity in the chloroform phase when compared with the control (Table 1). The radiochromatograms of the chloroform phase showed only one major radioactive spot with an R_F value corresponding to that of nicotine (Text-fig. 1). Some activity always remained at the origin but it amounted to only about 3% of the total activity in the chloroform phase.

The incubations with slices of the liver, which is known to be the main site of nicotine transformation (Hansson *et al.* 1964), were always run in parallel as reference

Table 1. *Distribution of ra*
of [^{14}C]nicotine with pancre
extraction with chloroform
480000 c.p.m. in each flask

T
Control
Pancreas
Liver

Pancreas

Text-fig. 1. Autoradiogra
metabolites extracted in t
pancreas slices, liver slices
benzene:conc. NH_4OH (5:
Only the liver slices show
(R_F 0·35), γ-(3-pyridyl)-γ-o
(R_F 0·13), 'Y' (R_F 0·06) (fo

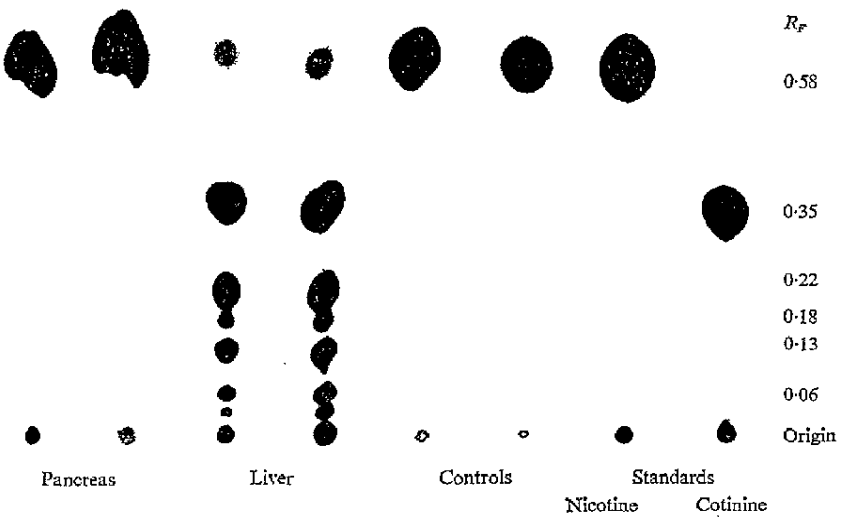
standards. The radiochro
the radioactivity was re
(Text-fig. 1), the major
cotinine. This result is in
by the mouse liver *in vitr*

Aut

The lack of nicotine-me
does not rule out the po

Table 1. *Distribution of radioactivity in various chemical fractions after the incubation of [^{14}C]nicotine with pancreatic and liver slices from mice (incubation time = 2 h) and extraction with chloroform:methanol (2:1, v/v) (3·4 µg [^{14}C]nicotine equivalent to 480000 c.p.m. in each flask)*

Tissue	Percentage of total radioactivity recovered: Chloroform phase	Water–methanol phase
Control (boiled liver slices)	99·0	1·0
Pancreas	98·8	1·2
Liver	89·3	10·7



Text-fig. 1. Autoradiogram of a thin-layer chromatogram, showing nicotine and nicotine metabolites extracted in the chloroform phase after incubation of [^{14}C]nicotine with mouse pancreas slices, liver slices and controls (boiled liver slices). Solvent system, ethanol:acetone: benzene:conc. NH_4OH (5:40:50:5, by vol.); solvent front, 12·5 cm; exposure time, 4 weeks. Only the liver slices show metabolism of nicotine. Components: nicotine (R_F 0·58), cotinine (R_F 0·35), γ-(3-pyridyl)-γ-oxo-*N*-methyl-butyramide (R_F 0·22), 'X' (R_F 0·18), hydroxycotinine (R_F 0·13), 'Y' (R_F 0·06) (for identification of components see Stålhandske, 1970).

standards. The radiochromatograms of the chloroform phase (where about 90 % of the radioactivity was recovered) showed several separate radioactive metabolites (Text-fig. 1), the major metabolite having an R_F value corresponding to that of cotinine. This result is in accordance with earlier studies on the metabolism of nicotine by the mouse liver *in vitro* (Hansson *et al.* 1964; Stålhandske, 1970).

Autoradiographic study with [^{14}C]cotinine

The lack of nicotine-metabolizing activity in the pancreatic slices observed *in vitro* does not rule out the possibility that in-vivo metabolites of nicotine can be trans-

ferred to the pancreas from other organs. The whole-body autoradiograms from the mice injected with the main nicotine metabolite cotinine, did not, however, show any specific accumulation of radioactivity in the pancreatic islets. The activity present in the pancreatic islets did not exceed the activity in the blood at any of the time intervals studied (Pl. 4, fig. 7). Neither could any specific accumulation of radioactivity be observed in the thyroid gland after the injection of [^{14}C]cotinine.

DISCUSSION

The results of the present investigation show that the injection of radioactive nicotine into mice leads to the accumulation of label in the cells of the pancreatic islets and in some cells of the thyroid, most probably identical with calcitonin-producing 'C' cells. Similarly, a high concentration of radioactivity can be seen in the calcitonin-producing UBG of chicks after the injection of [^{14}C]nicotine.

Administration of [^{14}C]cotinine, which in mice has been shown to be a principal metabolite of nicotine (Hansson & Schmiterlöw, 1962; Hansson *et al.* 1964; Stålhandske, 1970), did not result in any specific accumulation of radioactivity either in the pancreatic islets or in the thyroid. This, together with the results of metabolic studies which showed the inability of the mouse pancreas to metabolize [^{14}C]nicotine to any significant extent *in vitro*, indicates that the radioactivity observed in the endocrine organs studied represents mainly the unchanged nicotine.

The cells of the pancreatic islets as well as the calcitonin-producing cells of the UBG have been shown to receive parasympathetic and/or sympathetic innervation (Coupland, 1958; Libman & Sutherland, 1965; Cegrell, 1968; Hodges & Gould, 1969; Lever & Findlay, 1971). Since the resolution of the present autoradiograms does not permit differentiation between nerve fibres and cells, it cannot be excluded that in addition to the accumulation in the parenchymal cells there may also be an uptake in nerve endings.

As mentioned in the Introduction, nicotine has also been shown to be accumulated in other endocrine cell systems such as the hypophysis and the adrenal medulla (Hansson & Schmiterlöw, 1962). Like several polypeptide hormone-producing organs, the cells of pancreatic islets and the parafollicular cells of the thyroid as well as the ultimobranchial main cells possess the ability to concentrate and store certain monoamines such as dopamine and serotonin either endogenously or after the injection of precursors (Falck & Hellman, 1963; Falck *et al.* 1964; Ritzén *et al.* 1965; Larson, Owman & Sundler, 1966; Cegrell, 1968; Tjälve, 1971). In addition, they are known to show a positive reaction for pseudo- or true cholinesterase (Coupland, 1958; Carvalheira & Pearse, 1967; Pearse, 1969; Welsch & Pearse, 1969).

The accumulation of nicotine in the endocrine cell systems might indicate that nicotine could share common transport and/or storage mechanisms with biogenic amines in the cells.

Both the catecholamines and serotonin have been shown to effect directly the release of insulin in various animal species (Porte, 1964; Malaisse, Malaisse-Lagae, Wright & Ashmore, 1967; Telib, Raptis, Schröder & Pfeiffer, 1968; Feldman & Lebovitz, 1970; Feldman, Boyd & Lebovitz, 1971; Lundquist, 1971; Tjälve, 1971). The secretion of calcitonin could also be affected by catecholamines (Bates, Bruce &

Care, 1969; Philippo, Br
A stimulatory effect of ac
authors (Malaisse *et al.* 19
& Lebovitz, 1970). The pr
the calcitonin-producing
effect of this drug on the r

In in-vitro experiments
exert an effect on insulin r
inhibition of insulin secret
insulin secretion at a low c

An effect of nicotine on
ference with the biogenic
may be of importance in
nervous mechanisms have
(Kaneto, Kosaka & Nakao
effect via nerves may con

In the central nervous sy
of catecholamine, serotoni
1965; Armitage & Hall, 19
1972). The influence of nic
that of nicotine on insulin s
amines from the adrenal m
in response to nerve stim
reported to cause depletic
administered to rats in sr
Thompson, Spezia & Angul
in the enterochromaffin ce
belong to the cells of the an
1969).

The distribution of nicoti
seen after the injection of
ström, Sundwall, Ullberg &
& Tjälve, 1972; P. Slanina,
Like nicotine, these drugs
the parafollicular cells of th
ability of the cells of the A
pine and local anaesthetics
might be considered to be
crine cells. All these drugs
different cell systems via a

The investigation was su
and Research Foundation),
bergs Stiftelse'.

Care, 1969; Philippo, Bruce & Lawrence, 1969; Ziegler, Delling & Pfeiffer, 1970). A stimulatory effect of acetylcholine on insulin secretion has been shown by several authors (Malaisse *et al.* 1967; Kaneto, Kajinuma, Kosaka & Nakao, 1968; Feldman & Lebovitz, 1970). The present localization of nicotine in the pancreatic islets and in the calcitonin-producing cells of mice and chicks might also indicate a possible effect of this drug on the release and/or storage of the corresponding hormones.

In in-vitro experiments with rabbit pancreatic pieces, nicotine has been found to exert an effect on insulin release (Tjälve & Popov, 1973). The effect was twofold with inhibition of insulin secretion at a high concentration of nicotine but stimulation of insulin secretion at a low concentration of nicotine.

An effect of nicotine on hormone storage and/or release might take place via interference with the biogenic amines, present within the hormone-producing cells, and may be of importance in the release of the hormones produced by the cells. Since nervous mechanisms have been shown to be of importance in the secretion of insulin (Kaneto, Kosaka & Nakao, 1967; Malaisse *et al.* 1967), it also seems possible that an effect via nerves may contribute to the nicotinic influence on insulin secretion.

In the central nervous system nicotine is known to cause changes in turnover rates of catecholamine, serotonin and acetylcholine both *in vivo* and *in vitro* (e.g. Pepeu, 1965; Armitage & Hall, 1967; Bhagat, Kramer & Seifter, 1967; Goodman & Weiss, 1972). The influence of nicotine on the adrenal medulla shows a similar pattern to that of nicotine on insulin secretion; small doses stimulate the secretion of catecholamines from the adrenal medulla, while large doses of nicotine prevent their release in response to nerve stimulation (Volle & Koelle, 1970). Nicotine has also been reported to cause depletion of serotonin from the gastrointestinal mucosa when administered to rats in small, but not in high concentrations (Thompson, 1968; Thompson, Spezia & Angulo, 1969). Most of the gastrointestinal serotonin is stored in the enterochromaffin cells (see Håkansson, 1970) which are also considered to belong to the cells of the amine precursor uptake and decarboxylation series (Pearse, 1969).

The distribution of nicotine observed in the present investigation is similar to that seen after the injection of atropine and some local anaesthetics (Albanus, Hammarström, Sundwall, Ullberg & Vangbo, 1968; Ullberg & Hammerström, 1969; Slanina & Tjälve, 1972; P. Slanina, H. Tjälve, J. Hammerström & S. Ullberg, in preparation). Like nicotine, these drugs have been shown to accumulate in the pancreatic islets, the parafollicular cells of the thyroid, the hypophysis and the adrenal medulla. The ability of the cells of the APUD group to accumulate drugs, such as nicotine, atropine and local anaesthetics, which influence adrenergic or cholinergic mechanisms, might be considered to be yet another common characteristic shared by these endocrine cells. All these drugs may affect the release of the hormones produced by the different cell systems via an influence on the biogenic amines in the cells.

The investigation was supported by the American Medical Association (Education and Research Foundation), the Swedish Tobacco Co. and 'Ragnar and Torsten Söderbergs Stiftelse'.

REFERENCES

Albanus, L., Hammarström, L., Sundwall, A., Ullberg, S. & Vangbo, B. (1968). Distribution and metabolism of ^{3}H-atropine in mice. *Acta physiol. scand.* **73**, 447–456.

Almquist, S., Malmquist, E., Owman, C., Ritzén, M., Sundler, F. & Swedin, G. (1971). Dopamine synthesis and storage, calcium-lowering activity, and thyroidal properties of chicken ultimobranchial cells. *Gen. comp. Endocr.* **17**, 512–525.

Appelgren, L. E., Hansson, E. & Schmiterlöw, C. G. (1963). Localization of radioactivity in the superior cervical ganglion of cats following injection of ^{14}C-labelled nicotine. *Acta physiol. scand.* **59**, 330–336.

Armitage, A. K. & Hall, H. G. (1967). The effects of nicotine on the electrocorticogram and spontaneous release of acetylcholine from the cerebral cortex of the cat. *J. Physiol., Lond.* **191**, 115*P*–116*P*.

Bates, R. F. L., Bruce, J. B. & Care, A. D. (1969). The effect of catecholamines on calcitonin secretion in the pig. *J. Endocr.* **46**, xi–xii.

Berlin, M. & Ullberg, S. (1963). Accumulation and retention of mercury in the mouse. *Archs envir. Hlth* **6**, 589–601.

Bhagat, B., Kramer, S. Z. & Seifter, J. (1967). The effects of nicotine and other drugs on the release of injected ^{3}H-norepinephrine and on endogenous norepinephrine levels in the rat brain. *Eur. J. Pharmacol.* **2**, 234–235.

Brown, D. A., Hoffmann, P. C. & Roth, L. J. (1969). ^{3}H-Nicotine in cat superior cervical and nodose ganglia after close-arterial injection *in vivo*. *Br. J. Pharmac.* **35**, 406–417.

Bussolati, G. & Pearse, A. G. E. (1967). Immunofluorescent localization of calcitonin in the 'C' cells of pig and dog thyroid. *J. Endocr.* **37**, 205–209.

Carvalheira, A. F. & Pearse, A. G. E. (1967). Comparative cytochemistry of C cell esterases in the mammalian thyroid–parathyroid complex. *Histochemie* **8**, 175–182.

Cegrell, L. (1968). The occurrence of biogenic monoamines in the mammalian endocrine pancreas. *Acta physiol. scand.* Suppl. **314**, 1–60.

Copp, H. D. (1969). Endocrine control of calcium homeostasis. *J. Endocr.* **43**, 137–161.

Coupland, R. E. (1958). The innervation of pancreas of the rat, cat, and rabbit as revealed by the cholinesterase technique. *J. Anat.* **92**, 143–149.

Falck, B. & Hellman, B. (1963). Evidence for the presence of biogenic amines in pancreatic islets. *Experientia* **19**, 139–140.

Falck, B., Larsson, B., von Mecklenburg, C., Rosengren, E. & Svenaeus, K. (1964). On the presence of a second specific cell system in mammalian thyroid gland. *Acta physiol. scand.* **62**, 491–492.

Feldman, J. M., Boyd, A. E. & Lebovitz, H. E. (1971). Structural determinants of catecholamine action on in-vitro insulin release. *J. Pharmac. exp. Ther.* **176**, 611–621.

Feldman, J. M. & Lebovitz, H. E. (1970). Serotonin inhibition of in-vitro insulin release from golden hamster pancreas. *Endocrinology* **86**, 66–70.

Garlich, J. D. (1971). A technique for the surgical removal of the ultimobranchial glands from the domestic fowl. *Poultry Sci.* **50**, 700–702.

Goodman, F. R. & Weiss, G. B. (1972). Effects of nicotine on uptake and metabolism of ions and serotonin in slices obtained from different brain areas. Abstracts circulated at *Fifth Int. Congr. Pharmacol., San Francisco, U.S.A.* 23–28 *July* 1972, p. 85.

Håkansson, R. (1970). New aspects of the formation and function of histamine, 5-hydroxytryptamine and dopamine in gastric mucosa. *Acta physiol. scand.* Suppl. **340**, 7–113.

Hammarström, L., Appelgren, L. E. & Ullberg, S. (1965). Improved method for light microscopy autoradiography with isotopes in water-soluble forms. *Expl Cell. Res.* **37**, 608–613.

Hansson, E., Hoffman, P. C. & Schmiterlöw, C. G. (1964). Metabolism of nicotine in mouse tissue slices. *Acta physiol. scand.* **61**, 380–392.

Hansson, E. & Schmiterlöw, C. G. (1962). Physiological disposition and fate of ^{14}C-labelled nicotine in mice and rats. *J. Pharmac. exp. Ther.* **137**, 91–102.

Hirsch, P. F. & Munson, P. L. (1969). Thyrocalcitonin. *Physiol. Rev.* **49**, 548–822.

Hodges, R. D. & Gould, R. P. (1969). Partial nervous control of the avian ultimobranchial body. *Experientia* **25**, 1317–1319.

Kaneto, A., Kosaka, K. & Nakao, K. (1967). Effects of stimulation of the vagus nerve on insulin secretion. *Endocrinology* **80**, 530–536.

Kaneto, A., Kajinuma, H., Kosaka, K. & Nakao, K. (1968). Stimulation of insulin secretion by parasympathomimetic agents. *Endocrinology* **83**, 651–658.

Kracht, J., Hachmeister, V., Brevstedt, H. J., Bönicke, J. & Lenke, M. (1968). Histopathological investigations on C cells. *Proc. Symp. Thyrocalcitonin and C cells*, 1968, pp. 143–151. Ed. S. Taylor. London: Heinemann.

Larson, B., Owman, C. & Sundler, F. (1966). Monoaminergic mechanisms in parafollicular cells of the mouse thyroid gland. *Endocrinology* **78**, 1109–1114.

Lever, J. D. & Findlay, J. A. (1
creatic cells. In *Subcellular or*
& K. Lederis. Cambridge Uni

Libman, C. J. & Sutherland, S. I
J. Anat. **98**, 420–421.

Lundquist, I. (1971). Insulin se
physiol. scand. Suppl. **372**, 9–

McKennis, H., Jr. (1960). The e

Malaisse, W., Malaisse-Lagae, F.
gic agents on insulin secretio

Pearse, A. G. E. (1969). The sy
of the APUD series and the
J. Histochem. Cytochem. **17**, 3

Pepeu, G. (1965). Nicotina e ace

Philippo, M., Bruce, J. B. & La
conscious sheep. *J. Endocr.* **4**

Porte, D., Jr. (1964). Sympathet

Ritzén, M., Hammarström, L. &
mine and 5-hydroxytryptopha

Slanina, P. & Tjälve, H. (1972).
of the thyroid in mice shown

Smith, A. D. & Winkler, H. (196
of bovine adrenal medulla. *Bi*

Stahl, E. (1962). *Dünnischicht-C*

Stålhandske, T. (1970). The me
physiol. scand. **78**, 236–248.

Stålhandske, T., Slanina, P., Tj
of ^{14}C-nicotine in livers of foet

Telib, M., Raptis, S., Schröder,
Diabetologia **4**, 253–256.

Thompson, J. H. (1968). The eff
332.

Thompson, J. H., Spezia, Ch. A
nicotine. *J. Am. med. Ass.* **20**

Tjälve, H. (1971). Catechol- an
histochemical and radioimmu

Tjälve, H. & Popov, D. (1973). E
pancreas pieces. *Endocrinology*

Tjälve, H. & Slanina, P. (1971).
studied by combined autor
mikrosk. Anat. **113**, 83–93.

Ullberg, S. (1954). Studies on th
radiol. Suppl. **118**, 1–110.

Ullberg, S. (1958). Autoradiogra
U.N. int. Conf. Peaceful Uses
Nations.

Ullberg, S. & Hammarström, L.
topes in Pharmacology, pp. 225

Volle, R. L. & Koelle, G. B. (197
basis of therapeutics, pp. 585–6
Company.

Welsch, U. & Pearse, A. G. E. (
chemie **17**, 1–10.

Ziegler, R., Delling, G. & Pfeiffer,
gland of the hen. *Calcitonin.*
Heinemann.

Lever, J. D. & Findlay, J. A. (1971). The functional significance of the specific granule content of β-pancreatic cells. In *Subcellular organization and function in endocrine tissues*, pp. 437–456. Eds H. Heller & K. Lederis. Cambridge University Press.

Libman, C. J. & Sutherland, S. D. (1965). An investigation into the intrinsic innervation of the pancreas. *J. Anat.* **98**, 420–421.

Lundquist, I. (1971). Insulin secretion. Its regulation by monoamines and acid amyloglucosidase. *Acta physiol. scand.* Suppl. **372**, 9–37.

McKennis, H., Jr. (1960). The excretion and metabolism of nicotine. *Ann. N.Y. Acad. Sci.* **90**, 36–42.

Malaisse, W., Malaisse-Lagae, F., Wright, P. H. & Ashmore, J. (1967). Effects of adrenergic and cholinergic agents on insulin secretion *in vitro*. *Endocrinology* **80**, 975–978.

Pearse, A. G. E. (1969). The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. *J. Histochem. Cytochem.* **17**, 303–313.

Pepeu, G. (1965). Nicotina e acetilcolina cerebrale. *Archo ital. Sci. farmac.* **15**, 93–94.

Philippo, M., Bruce, J. B. & Lawrence, C. B. (1969). The effect of adrenaline on calcitonin secretion in conscious sheep. *J. Endocr.* **46**, xii–xiii.

Porte, D., Jr. (1964). Sympathetic regulation of insulin secretion. *Archs intern. Med.* **123**, 252–260.

Ritzén, M., Hammarström, L. & Ullberg, S. (1965). Autoradiographic distribution of 5-hydroxytryptamine and 5-hydroxytryptophan in the mouse. *Biochem. Pharmac.* **14**, 313–321.

Slanina, P. & Tjälve, H. (1972). Uptake of ^{3}H-atropine in the pancreatic islets and the parafollicular cells of the thyroid in mice shown by microautoradiography. *Acta physiol. scand.* **84**, 254–260.

Smith, A. D. & Winkler, H. (1967). Purification properties of an acidic protein from chromaffin granules of bovine adrenal medulla. *Biochem. J.* **103**, 483–490.

Stahl, E. (1962). *Dünnschicht-Chromatographie*. Berlin–Göttingen–Heidelberg: Springer-Verlag.

Stålhandske, T. (1970). The metabolism of nicotine and cotinine by a mouse liver preparation. *Acta physiol. scand.* **78**, 236–248.

Stålhandske, T., Slanina, P., Tjälve, H., Hansson, E. & Schmiterlöw, C. G. (1969). Metabolism *in vitro* of ^{14}C-nicotine in livers of foetal, newborn and young mice. *Acta pharmac. tox.* **27**, 363–380.

Telib, M., Raptis, S., Schröder, K. E. & Pfeiffer, E. F. (1968). Serotonin and insulin release *in vitro*. *Diabetologia* **4**, 253–256.

Thompson, J. H. (1968). The effect of nicotine on intestinal serotonin levels. *Eur. J. Pharmacol.* **2**, 329–332.

Thompson, J. H., Spezia, Ch. A. & Angulo, M. (1969). The release of intestinal serotonin in rats by nicotine. *J. Am. med. Ass.* **207**, 1883–1886.

Tjälve, H. (1971). Catechol- and indolamines in some endocrine cell systems. An autoradiographical, histochemical and radioimmunological study. *Acta physiol. scand.* Suppl. **360**, 5–122.

Tjälve, H. & Popov, D. (1973). Effect of nicotine and nicotine metabolites on insulin secretion from rabbit pancreas pieces. *Endocrinology*. (In Press.)

Tjälve, H. & Slanina, P. (1971). Uptake of DOPA and 5-HTP in pancreatic islets and parafollicular cells studied by combined autoradiographic and fluorescence microscopic techniques. *Z. Zellforsch. mikrosk. Anat.* **113**, 83–93.

Ullberg, S. (1954). Studies on the distribution and fate of ^{35}S-labelled benzyl-penicillin in the body. *Acta radiol.* Suppl. **118**, 1–110.

Ullberg, S. (1958). Autoradiographic studies on the distribution of labelled drugs in the body. *Proc. 2nd U.N. int. Conf. Peaceful Uses of Atomic Energy, Geneva, September*, 1958, **24**, 248–254. Geneva: United Nations.

Ullberg, S. & Hammarström, L. (1969). Localization in relation to action. *Int. Conf. on Radioactive Isotopes in Pharmacology*, pp. 225–241. Eds P. G. Waser & B. Glasson. Chichester: Wiley-Interscience.

Volle, R. L. & Koelle, G. B. (1970). Ganglionic stimulating and blocking agents. In *The pharmacological basis of therapeutics*, pp. 585–600. Eds L. S. Goodman & A. Gilman. London, Toronto: The Macmillan Company.

Welsch, U. & Pearse, A. G. E. (1969). Electron cytochemistry in thyroid and parathyroid C cells. *Histochemie* **17**, 1–10.

Ziegler, R., Delling, G. & Pfeiffer, E. F. (1970). The secretion of calcitonin by the perfused ultimobranchial gland of the hen. *Calcitonin. Proc. 2nd Int. Symp. London*, pp. 301–310. Eds S. Taylor. London: Heinemann.

DESCRIPTION OF PLATES

PLATE 1

Fig. 1. Detail of a whole-body autoradiogram of a mouse 5 min after an i.v. injection of [^{14}C]nicotine. Note the accumulation of radioactivity (light areas) in the pancreatic islets. A high concentration of radioactivity can also be seen in the kidney.

Fig. 2. Detail of a whole-body autoradiogram of a mouse 30 min after an i.v. injection of [^{14}C]nicotine. An uptake of radioactivity (light areas) can be seen in the pancreatic islets. A high concentration of radioactivity is also present in the liver and the kidney.

PLATE 2

Fig. 3. Microautoradiogram of a pancreatic islet of a mouse 30 min after an i.v. injection of [^{3}H]nicotine. The activity is distributed throughout the whole islet. Ilford K2 nuclear plate. Haematoxylin–eosin. (×380.)

PLATE 3

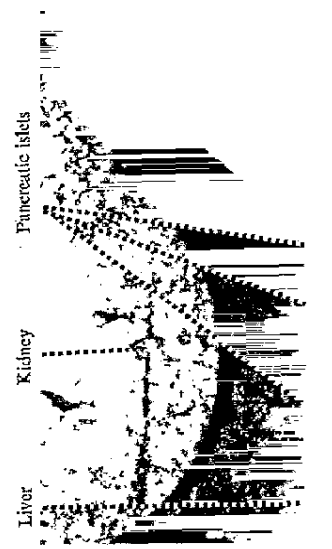
Fig. 4. Detail of a whole-body autoradiogram of a mouse 30 min after an i.v. injection of [^{14}C]nicotine. There is a high concentration (black grains) of radioactivity in some spots scattered all over the thyroid gland.

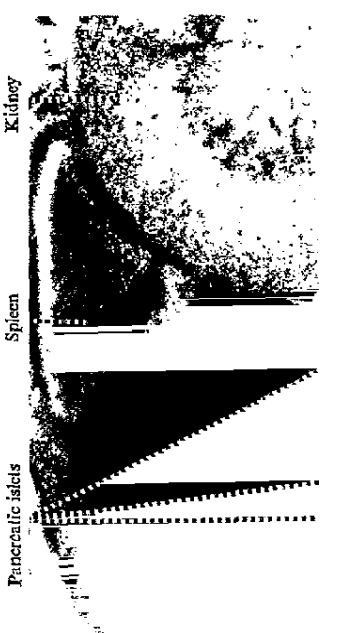
Fig. 5. Microautoradiogram of a thyroid of a mouse 30 min after an i.v. injection of [^{3}H]nicotine. The strongest labelling can be seen in some cells situated in or between the follicular walls, probably the parafollicular cells. Ilford G5 nuclear plate. Haematoxylin–eosin. (×400.)

PLATE 4

Fig. 6. Detail of a whole-body autoradiogram of a male chick 30 min after an i.v. injection of [^{14}C]nicotine. A high concentration of radioactivity is present in the ultimobranchial body. The thyroid shows a very low level of radioactivity. A comparison with the isotope staircase (top of the figure), where each step represents a double amount of radioactivity, shows that the radioactivity in the ultimobranchial gland exceeds that of the thyroid by 2–4 times.

Fig. 7. (*a*) Detail of a whole-body autoradiogram of a mouse 5 min after an i.v. injection of [^{14}C]cotinine. (*b*) A corresponding section stained with haematoxylin–eosin. The radioactivity in the pancreatic islets does not exceed the radioactivity in the exocrine pancreas or in the blood of the blood-vessel.





PREMYSL SLANINA AND H

i.v. injection of [^{14}C]nicotine.
lets. A high concentration of

i.v. injection of [^{14}C]nicotine.
lets. A high concentration of

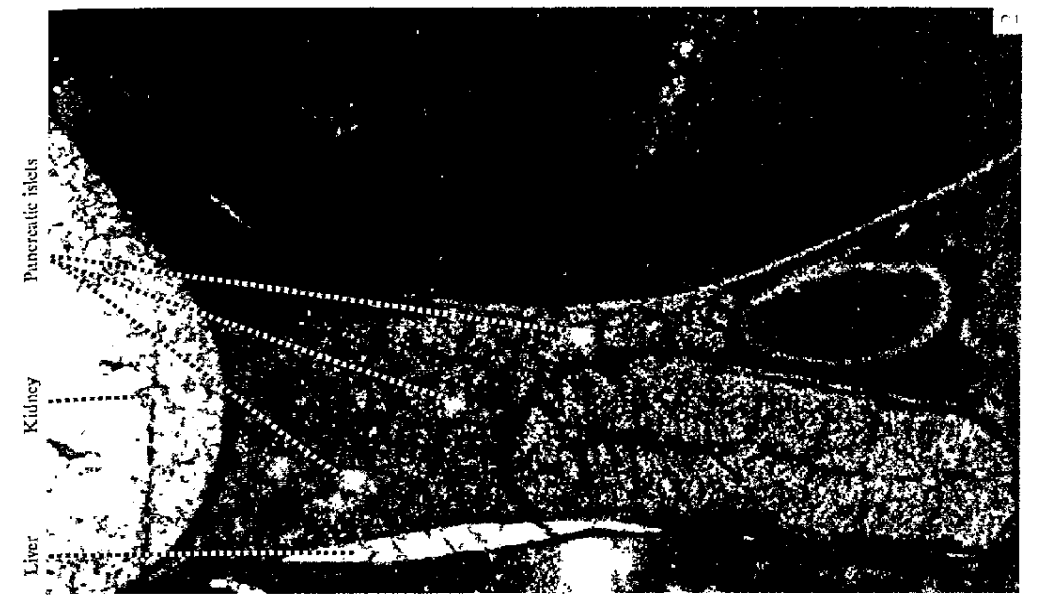
i.v. injection of [^{3}H]nicotine.
plate. Haematoxylin–eosin.

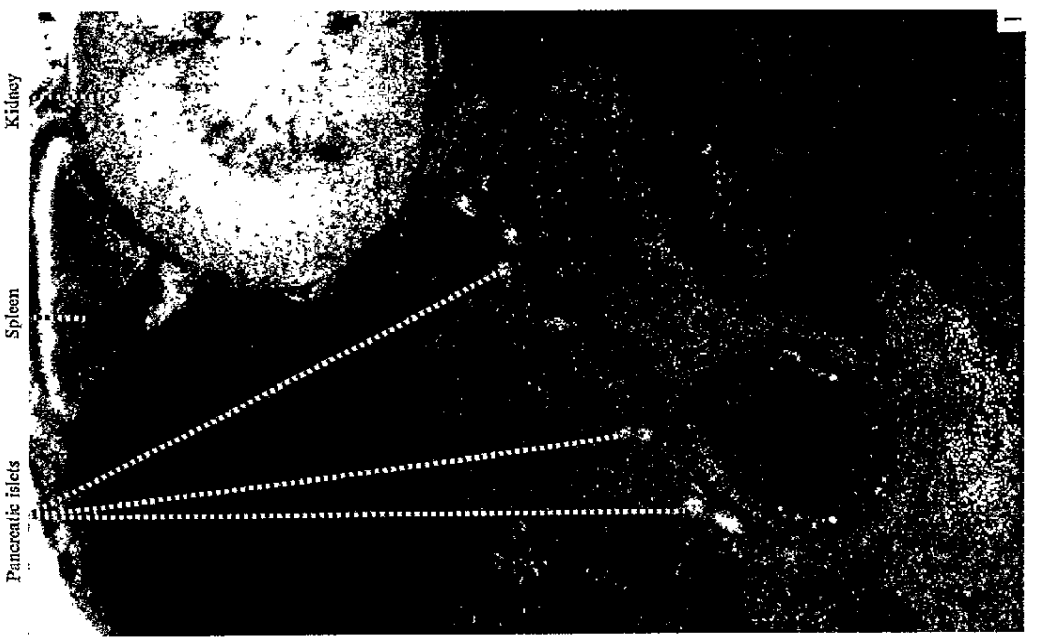
i.v. injection of [^{14}C]nicotine.
scattered all over the thyroid

njection of [^{3}H]nicotine. The
ılar walls, probably the para-

i.v. injection of [^{14}C]nicotine.
ly. The thyroid shows a very
the figure), where each step
in the ultimobranchial gland

i.v. injection of [^{14}C]cotinine.
ivity in the pancreatic islets
of the blood-vessel.





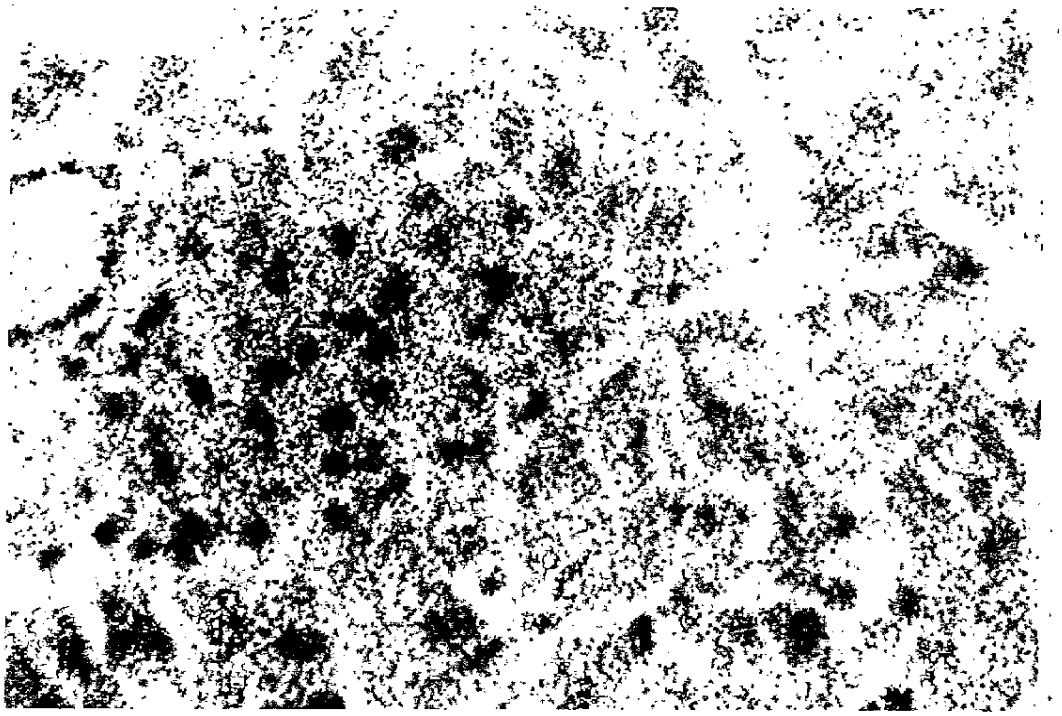


Figure 3

Journal of Endocrinc

PREMYSL SLANINA AND HA

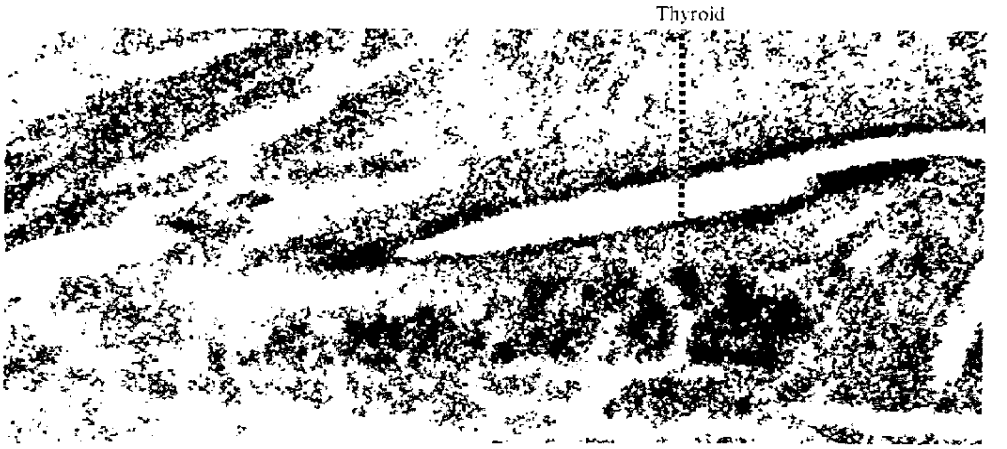


Figure 4

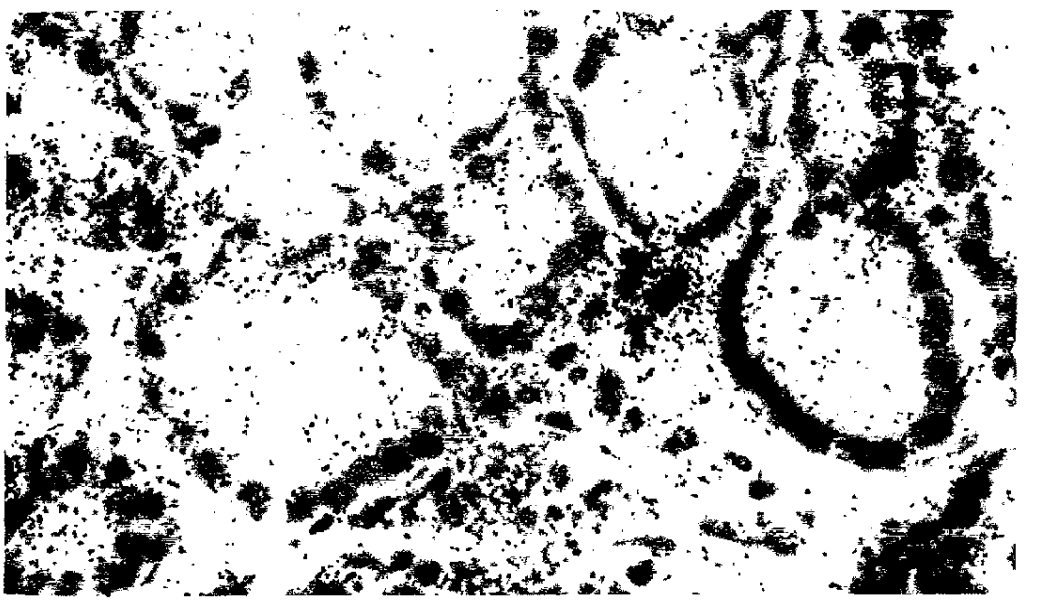


Figure 5

J. Endocr. (1973), 58, 31–39
Printed in Great Britain

EVID
BETWEEN PROLA
RISE IN MIL

C.
Depa
Un
J

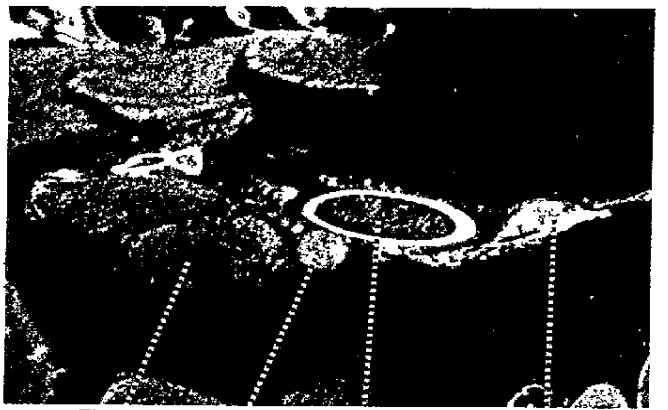


Figure 6

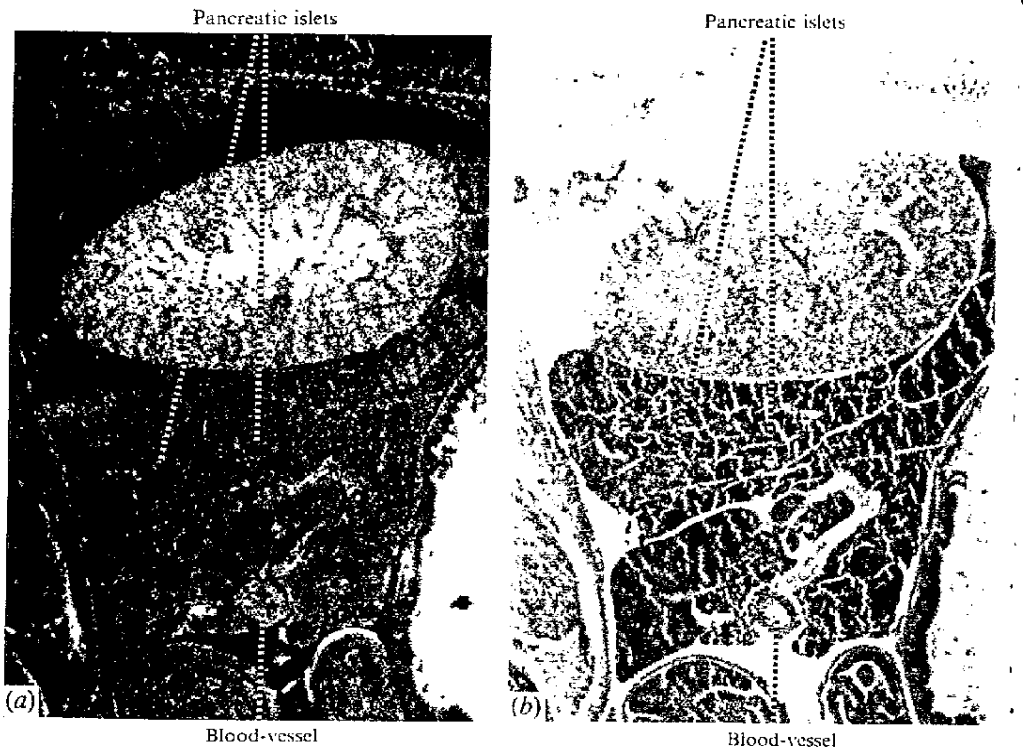


Figure 7

The mammary glan
room on day 14 *post*
day 21 *post partum*,
withdrawn the milk f
injections to the moth
or not the release of
injection of an extra
glands of rats not i
near capacity within
to refill any further.
lated to refill more c
vided prolactin either
the mothers to exter
8-h period of non-suc
plete mammary refilli
8-h period of non-su
The results of these i
suckling at the time
that milk which reac
16th h following suck
8 h appeared to be i
suckling.

The results suggest
from the time of relea
effect of the hormon
secreted after emptyi
result of prolactin r
response to exterocep

* Guest Investigator at the U
Investigaciones Biomedicas, Uni
D.F.